UNIVERSTY OF GONDAR

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES

DEPARTEMENT OF MEDICAL MICROBIOLOGY

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ENTROBACTERIACEAE IN UNIVERSITY OF GONDAR REFERRAL HOSPITAL ENVIRONMENTS, NORTHWEST ETHIOPIA

BY

TIGIST ENGDA (BSc., MSc. Candidate)

ADVISORS

FELEKE MOGES (MSc., PhD)

ASCHALEW GELAW (BSc., MSc.)

A THESIS SUBMITTED TO THE DEPARTEMENT OF MEDICAL MICROBIOLOGY, SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES, COLLEGE OF MEDICINE AND HEATLH SCIENCES, UNIVERSITY OF GONDAR, FOR PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCES IN MEDICAL MICROBIOLOGY.

JUNE 2014

GONDAR, ETHIOPIA

UNIVERSTY OF GONDAR

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES

DEPARTEMENT OF MEDICAL MICROBIOLOGY

A THESIS SUBMITTED TO THE DEPARTEMENT OF MEDICAL MICROBIOLOGY, SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES, COLLEGE OF MEDICINE AND HEATLH SCIENCES, UNIVERSITY OF GONDAR FOR PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCES IN MEDICAL MICROBIOLOGY.

Name of investigator	Tigist Engda(BSc.)
Names of Advisors	Feleke Moges (MSc., PhD) Aschalew Gelaw (BSc., MSc.)
Full title of the research	PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ENTROBACTERICEAE IN UNIVERSTY OF GONDAR REFERRAL HOSPITAL ENVIRONMENT
Duration of the research	January-June/2014
Study area	University of Gondar Referral Hospital environment
Total cost of the research	22,006.00 birr
Address of the investigator	Email : tigiengda@gmail.com Phone : +251 918 769447

JUNE 2014

GONDAR, ETHIOPIA

CERTIFICATE

This is to certify that the thesis entitled **"Prevalence and antimicrobial susceptibility patterns of extended spectrum beta lactamase producing entrobacteriaceae in University of Gondar Referral Hospital environments"** submitted by Tigist Engda for the award of MSc. Degree in Medical Microbiology was carried out under our supervision and the thesis has not been previously submitted in part or full for any degree or diploma at this or any another University.

Advisors	Signature	Date
1. Dr. Feleke Moges	----------	----------
2. Mr. Aschalew Gelaw	----------	----------

Examiners

1. **----------**

2. **----------**

DECLARATION

The research work in this thesis entitled "D**etection and Antimicrobial susceptibility pattern of o extended spectrum beta-lactamase producing entrobacteriaceae in the Gondar Referal Hospital environment, North west Ethiopia**'' was carried out by me under the supervision of Dr. Feleke Moges and Mr.Aschalew Gelaw in the College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences, Department of Medical Microbiology University of Gondar, for the award of MSc Degree in Medical Microbiology. I declare that this work is original and has not been submitted to any other University or institution.

Place: Gondar, Ethiopia

Date: 13/6/2014

Name of the student: TIGIST ENGDA**.** **Signature** ----------

Advisors **Signature**

1. Dr. Feleke Moges (MSC.,PhD) ----------

2. Aschalew Gelaw (BSC., MSC**.)** ----------

Examiners

1. ----------

2. ----------

ACKNOWLEDGEMENTS

It gives me great pleasure to thank my advisors, Dr.Feleke Moges and Mr.Aschalew Gelaw from the Department of Medical Microbiology, University of Gondar, for giving me their constructive comments to develop this thesis. They spent their precious time in correcting, guiding and shaping this research work. Without their valuable advice, the accomplishment of this thesis would have been impossible.

I would like to thank the Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar for giving me this opportunity.

Table of Contents

.

LIST OF TABLE

LIST OF ABERIVATION

CTX-M Cefotaximase, Munich

CLSI Clinical laboratory Standard Institute

DDST Double-Disc Synergy Test

ESBL Extended Spectrum Beta Lactamase

HAIs Health Care Associated Infections

ICU Intensive Care Unit

MIC Minimum Inhibitory Concentration

OXA Oxacillin

SHV Sulphydryl Variable

SPSS Statistical Package for Social Sciences

TEM Temoneira

UTI Urinary Tract Infection

ABSTRACT

Introduction: Entrobacteriaceae is a large family of gram-negative bacteria and common cause of nosocomial infection. Beta–lactam antibiotics are the possible drugs for infections caused by entrobacteriaceae. But they produce extended spectrum beta-lactamases that cause high resistance to the beta-lactam antibiotics.

Objective: To assess the magnitude of extended spectrum beta-lactamase producing entrobacteriaceae in Gondar Referral Hospital environment.

Methods: A cross sectional study was carried out at the University of Gondar Referral Hospital from January to June 2014. A total of 384 environmental samples were taken from inanimate objects and waste water from sewage. The samples were cultured onto MacConkey agar, then species was identified by biochemical tests and isolates were also subcultured on to Hicrome ESBL agar base to assess ESBL production. Antimicrobial susceptibility tests were done using the disc diffusion method. Data was entered in SPSS version 20 statistical software and analysed. The result was presented in table, figures and text.

Result: From a total of 384 samples, 163 (42.45%) entrobacteriaceae were isolated and of which 57(35.0%) were ESBL producing entrobacteriaceae. The most common ESBL producing isolates were *Klebsiella pneumoniae 24 (*14.7%) followed by *Escherchia coli* 20 (12.3%) and *Proteus mirabilis* (2.4%). Most of ESBL producing isolates were from waste water 14 (24.6%), sink 13 (22.8%) and bed side table 13(22.8). All ESBL producing entrobacteriaceae were resistant to ceftriaxone, ceftazidime, cefpirome, cefpodoxime and augmentin. Also resistant to chloramphenicol, 40(70.2%), cotrimoxazole, 37(64.9%), norfloxacilin, 24(42.1%), ciprofloxacilin 25(43.9%), gentamicin, 11(19.1%).

Conclusion: ESBL producing entrobacteriaceae were isolated from hospital environments. *Klebsiella pneumoniae*, *Escherchia coli* & *Proteus mirabilis* were predominant ESBL producing. It is important that health professional and other workers should take an active role in infection control and encourage good antibiotic prescribing practice.

Key words: ESBL, Entrobacteriaceae, Hospital Environment

1. INTRODUCTION

Hospital environment is a potential source of nosocomial infections, since it houses both patients with diverse pathogenic microorganism and large number of susceptible individuals. Nosocomial infections are infections that have been caught in hospital and are potentially caused by organisms that are resistant to multiple antimicrobial agents. The emergence of multi-drug resistant organisms in hospital results in a problem to treat nosocomial infections (1).

The ever-increasing bacterial resistance to antibiotics is one of the most challenging tasks of all the medical issues today (2). Persistent exposure of the bacterial strains to multitude of beta-lactamases has induced a dynamic and continuous production and mutation of beta-lactamases in the bacteria. This leads to the development of extended spectrum beta-lactamases (ESBLs) which cause resistance to broad spectrum beta-lactam antibiotics. This resistance mechanism has been responsible for nosocomial as well as community out breaks and cause of serious therapeutic failure (3).

Extended spectrum beta-lactamase is plasmid-mediated enzymes hydrolysing most penicillins and cephalosporins, including oxyimino-beta-lactam compounds (cefuroxime, third and fourth-generation cephalosporin and aztreonam) but not cephamycins and carbapenems. The Ambler molecular classification and the Bush-Jacoby-Medeiros functional classification are the two most commonly used classification systems for β-lactamases. Ambler scheme divides β-lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino acid similarity) and not phenotypic characteristics. In the Ambler classification scheme, β-lactamases of classes A, C and D are serine β-lactamases. In contrast, the class B enzymes are metallo-β-lactamases. With the exception of OXA-type enzymes (which are class D enzymes), the ESBLs are of molecular class A. Most ESBLs belong to the Ambler class A of beta-lactamases and are inhibited by beta-lactamase inhibitors (clavulanate, sulbactam and tazobactam) (3, 4).

According to National Committee for Clinical Laboratory Standards (NCCLS) now Clinical and Laboratory Standards (CLSI) interpretive definitions of ESBLs do not always increase MICs to levels characterized as resistant. Now, it is mandatory that the routine clinical microbiology laboratory employs ESBL detection methods which are sensitive enough to recognize the level of resistance that would be achieved by the

situation given in vivo. The methods for detection of ESBLs can be broadly divided into two groups: phenotypic methods that use non-molecular techniques, which detect the ability of the ESBL enzymes to hydrolyse different cephalosporins; and genotypic methods, which use molecular techniques to detect the gene responsible for the production of the ESBL. Clinical diagnostic laboratories use mostly phenotypic methods because these tests are easy to do, are cost effective, and have been incorporated in most automated susceptibility systems, making them widely accessible. However, phenotypic methods are not able to distinguish between the specific enzymes responsible for ESBL production (SHV, TEM, and CTX-M types). The detection of ESBL-producing bacteria in laboratories is a crucial step for appropriate management of patients (4,5).

The first ESBL-producing strains were identified in 1983, and since then have been observed worldwide (4). This distribution has been due to the clonal expansion of producer organisms and the horizontal transfer of their genes. By far the most important groups of ESBLs are Cefotaximase, munich (CTX-M) enzymes, followed by Sulphydryl Variable (SHV) and Temoneira (TEM) derived ESBLs. Certain Oxacillin (OXA) derived enzymes are also included within ESBLs, although inhibition by class A-beta-lactamase inhibitors is weaker than for other ESBLs (5).

Rates of CTX-M infections have increased during the last decade compared with rates of TEM and SHV infections (6). These enzymes confer resistance to beta-lactam antibacterial drugs, particularly cephalosporin, and may be accompanied by co-resistance to drugs of other classes. Because of the ESBL resistance and associated co-resistance, empiric oral antibacterial therapy appears to be limited, especially in the community settings (7).

Extended spectrum beta lactamase production has been observed mostly in entrobacteriaceae and its resistance increased mainly due to the spreading of ESBL, and also different genes are emerged (8). Resistance genes of the ESBL type are mostly plasmid associated and therefore can spread among bacteria and it can lead to the overall distribution of antimicrobial resistance. This resistance increases morbidity and mortality in infected individuals by hampering the adequate provision of effective chemotherapy (9).

The most frequently encountered members of ESBL-producing entrobacteriaceae are *Escherichia coli*, *K. pneumoniae*, and *Proteus* spp. However, all other clinically relevant

entrobacteriaceae species are also common ESBL producers. The prevalence of ESBL positive isolates depends on a range of factors including species, geographic locality, hospital or ward, group of patients and type of infection, and large variations have been reported in different studies (10, 11).

Extended spectrum beta lactamase resistance pattern was initially considered as a problem related to nosocomial out break, mainly in Intensive Care Units (ICUs), that is, recent surgical procedures, use of catheters, bladder catheterization, long-term hospitalization and previous use of cephalosporins or amino glycosides (12). However, the distribution of ESBL producing entrobacteriaceae have been become very common in almost any other area of hospital which indicates high possibility of horizontal gene transfer among strains of different genera within the hospital environment (13,14,15).There is shortage of report about ESBL producing entrobacteriaceae at hospital environment in Northwest Ethiopia.

Therefore, the aims of this study were to assess and determine the rate of ESBLs producing entrobacteriaceae in the hospital environment at University of Gondar Referral Hospital.

2. LITERATURE REVIEW

2.1. Distribution of Entrobacteriaceae in inanimate objects of the hospital Environment

Different studies in various parts of the world had assessed the extents of entrobacteriaceae contamination in the hospital environment. A cross sectional study in Nigeria was conducted and samples were obtained from doctors, nurses, orderlies, patients, air and fomites like beds, cannula, oral thermometer and table. A total of 56 bacteria were isolated. Gram negative bacilli were one part of isolate and among these, *Escherichia coli* were the highest (4; 7.1%). Others were *Klebsiella pneumoniae* (3; 5.3%), *Proteus spp*. (2; 3.5%) and *Entrobacter aerogenes* (2; 3.5%) (16).

Another cross sectional study was conducted at the university of Gondar teaching hospital from November 2010 to February 2011, on a total of 220 samples. Out of these 142 swab samples were collected from various hospital environments such as medical devices, inanimate objects and air in different areas (56 located in the operating room, 42 in surgical and 44 in orthopaedic wards) and other samples were pus, nasal and hand swabs. In inanimate objects, 142(52.9%) organisms were isolated and 41(28.9%) were gram negative. Out of these, *Klebsiella species* 11(26.8%), *Escherichia coli* 10(24.3%), *Proteus species* 5(12.2%) and *Entrobacter* 4(9.8%) were common among gram negative isolates. *Citrobacter* 2(4.9%), *Serrattia* 1(2.4%) were the least isolated. The hospital environment that had the highest number of bacterial isolates was the operating rooms but orthopaedic ward did not show any bacterial growth even after 24 hours of incubation (17)

2.2. Distribution of extended spectrum beta–lactamase producing entrobacteriaceae in inanimate objects of the hospital environment

Beta-lactam antibiotics are the most common drugs against entrobacteriaceae infections and on the other hand the emergence of resistance to extended spectrum cephalosporin has become a major concern to see this beta-lactam resistance (2, 14).

A study in France had also evaluated surface contamination on five standardized sites surrounding patients who were infected or colonized with ESBL producing *Klebsiella* species, (n=48) or ESBL producing *E.coli* (n=46). Environmental contamination was significantly more likely in the rooms of *Klebsiella* species patients (31% of 48 rooms positive; 6% of 240 sites positive) vs *E.coli* patients (4% of 46 rooms positive, 1% of 230 sites).The result was only 52% of the ESBL producing isolates were identical to the patients in the room, suggesting survival of ESBL producing bacteria to the prior occupants in to the room (18).

Another study was done in Auckland, Newzeland to measure the difference in hospital contamination rates between ESBL-producing *Klebsiella pneumonia* (ESBL-KP) and *Escherichia coli* (ESBL-EC) and to identify risk factors for contamination of the hospital environment with these organisms. Samples were collected in 8 surfaces in the rooms and bathrooms of adult patients colonized or infected with ESBL-EC or ESBL-KP throughout their hospital stay, that is blood pressure cuff, the nurses call bell, the top of patients bed side cabinet, the patient's over bed tray table, the toilet seat, the hand rail next to the toilet, basin tap in the bathroom and the bathroom's inside door handles. The overall contamination rate was 3.4%(38/1104) and was significantly higher for ESBL-KP than ESBL-EC (5.4% versus 0.4%; $p < 0.0001$),and antimicrobial susceptibility profiles were consistent between corresponding patient and environmental isolates without exception (19) .

A study in Australia, bacteria was isolated from patients with Health Acquired Infections exhibiting resistance to beta-lactam antibiotics over a one month period in 2011. All sampled patients were in a 14-bed burns unit and the environmental sample sites included shower drains, sinks, trolleys, and door handles. It was found that identical strains

carrying the same resistance regions were present in both patients and the hospital environment suggesting Health Acquired Infections can arise from bacteria resident in the immediate surrounds (20).

Study in Algeria, which was done in the Intensive Care Unit of Tlemcen University Hospital to identify and screen ESBLs isolates by API 20E and DDST respectively from 19 October to 23 November 2008. The result of the study was 2 cefotaxime-resistant isolates (*K. pneumonia* and *Entrobacter cloaceae*) which were recovered from surfaces of the ICU. Minimum inhibitory concentrations (MIC) of cefotaxime and ceftazidime were from 64 to 512mg/dl and 4 to 128mg/dl respectively (21).

There was also another study in Upper Egypt to evaluate the rate of environmental contamination of extended spectrum beta lactamase and ESBL producing *Klebsiella pneumoniae* and *Eschericia coli* as well as the susceptibility to 13 antimicrobial agents. A total of 227 environmental isolates, *K.pneumoniae* (n=102) and *E.coli* (n=125) were recovered from750 samples of 6 different environmental surfaces. ESBL production was observed in 80 isolates, for overall prevalence of 56.25% with a predominance of K.pneumoniae (45/80), followed by E.coli prevalence of 43.75 with predominance of (35/80). The resistance rate was higher among ESBL producers than non ESBL producers. All of *K.pneumoniae* and *E.coli* ESBL producers were found resistance (100%) to cephalotin, ampicillin, cefataxine, cefuroxime, ceftriaxone and ceftazidime. The resistance rate (%) was higher in azetreonam (93.3 and 100) and ceftazidime (95.5 and 91.42) then gentamicin (84.4 and 42.8) ciprofloxacilin (77.7 and 68.5) followed by cotrimoxazole (46.6 and 60) of ESBL producing *Klebsiella pneumoniae* and *Eschericia coli* strains, respectively (22).

There was study in Egypt, the Zagazig University Hospitals, in paediatric department to determine extended spectrum beta-lactamase producing *Klebsiella pneumoniae* in Neonatal Intensive Care Unit. From 343 environmental cultures taken from potential sources of infection, 8 isolates (2.3%) of *K. Pneumonia* were identified; 4 from suction tubes, 2 from the incubators and 2 isolates from the hands of Health Care Workers (23).

2.3. Distribution of Entrobacteriaceae in waste water in hospital sewage system

Different studies were done in waste water for a reservoir of different organism which infects the general population in the community.

One cross-sectional study was conducted at Gondar from January – June 2012. The waste water was collected from hospital sewage system of different sections of the Gondar university teaching hospital and non –hospital environment. A total of 60 waste water samples were processed and 113 bacterial isolates were recovered. Out of these 65 (57.5%) were from hospital environment and 48 (42.5%) were non-hospital environment. The most frequently identified bacterium in hospital waste water was *Klebsiella spp*. 11(22.9%) , E. coli 5(10.4%) and *Citrobacter spp* 8(16.7%) (24).

2.4. Distribution of ESBL-producing entrobacteriaceae in waste water in hospital sewage system.

Waste water contains high amount of resistant bacteria strains and antibiotic residues at a concentration able to inhibit the growth of susceptible bacteria which introduces into the sewage system that come from hospital (25, 26).

A study in three hospitals in poland, ESBL producing Entrobacteriaceae was investigated in 63 sewage samples. In the group of 310 randomly sampled strains isolated from hospital effluents, 295 (95.2%), 253 (81.6%), and 228 (73.5%) isolated were resistant to cefotaxime, ceftazidime and cefpodoxime, respectively (27).

A study was also done in Alexander, Egypt, from December 2012 and April 2013. The result of the study shows the existence of ESBL producing entrobacteriaceae in waste water. Waste water samples were collected; one from influent waste water (raw sewage) going into the treatment plant and other one from the effluent waste water after treatment. The result was show existence of ESBL producing Entrobacteriaceae and it was slightly higher in influent (69.8%) than effluent flow (57.7%). A total of ESBLs producing *E.coli* (74 isolates) and *K. pneumonia* (13 isolates) were identified (23). The bacterial isolates show highest antimicrobial resistance rates to amoxacilin-clavulanic acid, piperacillin-tazobactam and trimethoprim/sulfamethoxazole (28).

3. SIGNIFICANCE OF THE STUDY

Extended-spectrum beta lactamase producing entrobacteriaceae are an increasing challenge for health care practitioners while they are fighting health care-associated infections (HAIs). They have been responsible for numerous outbreaks of infections throughout the world. The outcomes of clinical data indicate that ESBLs are clinically significant because of their resistant genes which are usually present in the hospital and can reach the environment which finally lead to limited therapeutic option (9).

However, there is limitation of study in the area which addresses this issue to the health care personnel and community. Therefore, this study tries to show the distribution of extended spectrum beta-lactamase producing entrobacteriaceae, to identify the dominant ESBL producing isolates and to determine resistant pattern of isolates to the commonly used antibiotics in the hospital which may be important as first hand information in infection control and rational use of drugs in the hospital.

4. OBJECTIVE

4.1. General Objective

- To assess the prevalence and antimicrobial susceptibility patterns of extended spectrum beta-lactamase producing entrobacteriaceae at the University of Gondar Hospital Environment, Gondar, Northwest Ethiopia.

4.2. Specific Objectives

- To determine the prevalence of extended spectrum beta lactamase producing entrobacteriaceae..
- To determine the antimicrobial susceptibility pattern of extended spectrum beta lactamase producing entrobacteriaceae to the commonly used antibiotics in the hospital.

.

5. MATERIAL AND METHODS

5.1. Study area and period

The study was conducted at the University of Gondar Referral Hospital from January to June 2014.University of Gondar Referral Hospital is found in Gondar town in Northwest Ethiopia. The town is located at 748 km away from Addis Ababa, the capital city of Ethiopia. The town is situated at an altitude of 2200 m above sea level and total annual rainfall of 1800mm and an average temperature ranging from 14^{o}c-28^{o}c. University of Gondar Referral Hospital provides surgical ,medical, paediatric, gynaecologic, obstetric and ophthalmologic services to the community of over 5 million inhabitants. The hospital consists of 19 wards with 465 beds and outpatient departments.

5.2. Study design

A hospital based cross sectional study.

5.3. Source of population

The source populations were inanimate objects and waste water from sewage system of the hospital.

5.4. Study population

The study population was taken from taken from environmental surfaces (bed frames, bed side tables, floors, walls, sinks, door handles, and waiting chairs) of the hospital which are directly related to patients and patient family. Hence the focused areas are the intensive care unit, emergency, orthopaedic room, surgery, delivery ward, Out Patient Department(OPD), Maternal and Child Health (MCH), laboratory and pharmacy dispensing area. Waste water in sewage system of the hospital environment and chairs in hospital cafe were part of the population of the study.

5.5. Sample Size and Sampling techniques

Sample size Determination

The sample size was determined by using single population proportion formula. The population proportion of a target population with a certain characteristics is 0.5 (50%), desired accuracy of 0.05 at 95% confidence interval (Z statics=1.96), then the sample size was

$$\mathbf{n= Z^2p\ (1\text{-}p)/d^{2,}}$$

$$n= \frac{(1.96)^2(0.5)\ (1\text{-}0.5)}{(0.05)^2}$$

$$= 384$$

These samples include surfaces of floors, bed frames, bed side tables, door handles, walls, sinks, waiting chairs in intensive care unit, emergency room, surgery, orthopaedic, delivery, laboratory room, pharmacy dispensing area and waste water from hospital sewage system.

Sampling Techniques

In the hospital, 26 sites were identified to collect environmental specimens. In order to set sample distribution per site, a factor 'K' was used to calculate proportion of populations in the selected hospital environment. Finally a simple random sampling technique was used to select the study population. However, the total number of waste water source was used as sample size of waste water because it's sample was very small. Total population to be sampled were 2508 and the actual sample size were 384.The "K" factor is used for the calculation used to get size of each type of sample from the total were calculated as 384/2508=0.15.

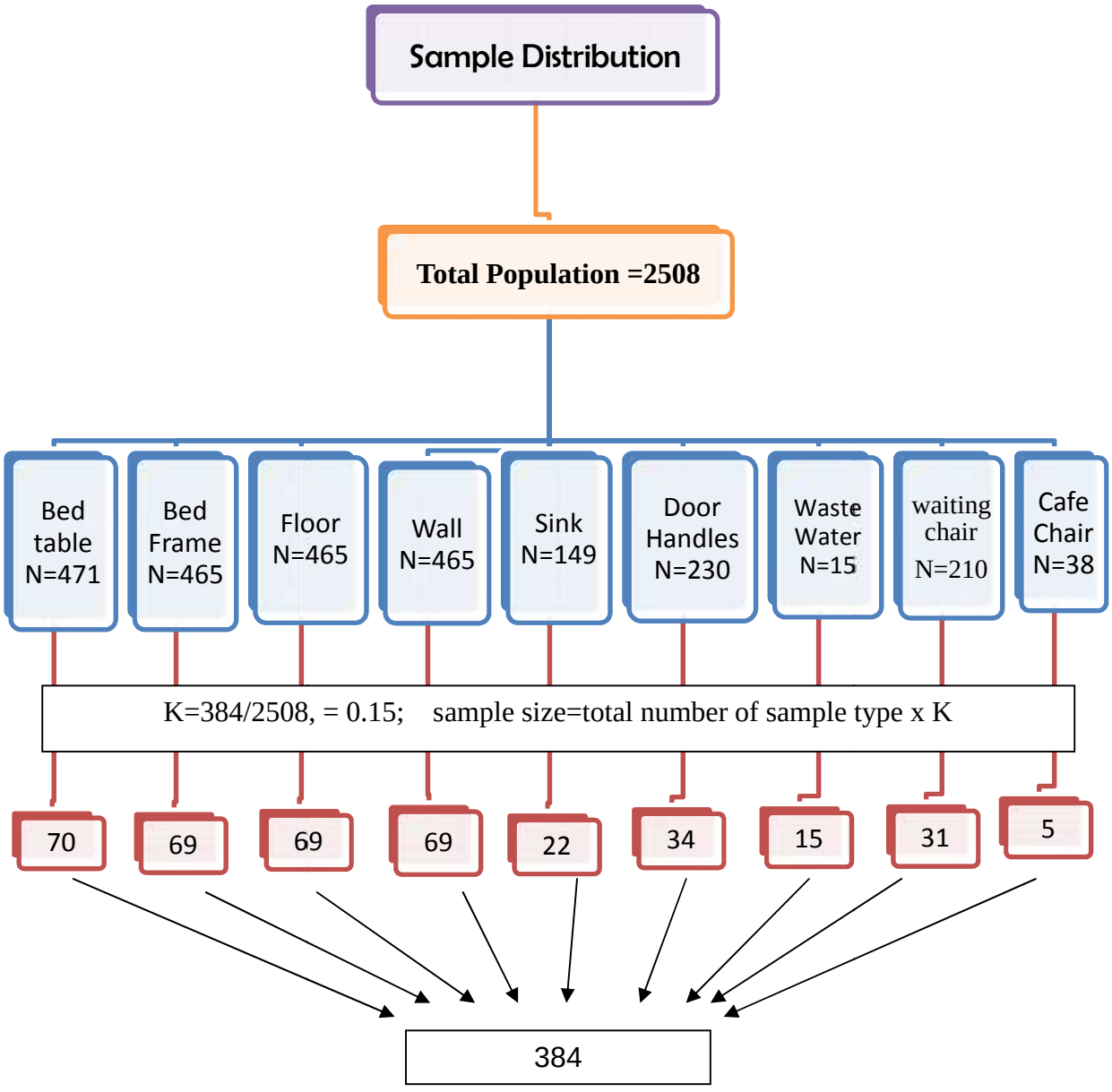

Figure: Schematic presentation of sampling procedure

5.6. Operational Definition

Extended Spectrum beta lactamase: - is an enzyme that hydrolyses the beta lactam ring of penicillins and cephalosporins, including oxyimino-beta-lactam compounds (cefuroxime, third and fourth-generation cephalosporins and aztreonam) but not cephamycins and carbapenemes.

Environmental swab: A swab specimen that is taken from inanimate objects from different section of the hospital.

Hospital environment: is the area of hospital which is allocated by individual (patients, non-patients), medical devices, bed frames, bed side tables, sections (wards), floor, walls, sinks, sewage, toilet, pipes waiting chair and cafe chair.

Hospital inanimate objects: is a material found in hospital for different activities, like, bed frames, bed side tables, floor, walls, sinks, sewage, toilet, pipes waiting chair and cafe chair.

Hospital sewage sample: is waste water in solution or suspension that is intended to be removed from different section of the hospital.

5.7. Study variable

Dependent variables

- Extended spectrum beta lactamase producing entrobacteriaceae
- Drug susceptibility pattern (susceptible, intermediate, resistance)

Independent variables

- Surfaces of floor, bed frames, bed side tables, door handles, walls, sinks, waste water, waiting chairs and hospital cafe chairs.

5.8. Data collection and processing

5.8.1. Specimen collection

A total of 384 environmental samples were collected from hospital inanimate objects and waste water from different hospital sewage system. Sterile cotton tipped swabs moistened with normal saline was rotated against the surfaces of in animate objects to obtain specimens. Samples were aseptically collected using sterile test tubes and sterile cotton swabs for surface sampling and waste water was collected from sewage system of the hospital by sterile screw capped bottle and was transported to Microbiology Laboratory. Each sample was transferred to the appropriate media within 2 hours (30).

5.8.2. Sample processing

5.8.2.1. Isolation

Swabs of surfaces and loop full of waste water were inoculated on MacConkey agar and the inoculated agar plates were incubated at 37^{o}c for 24 hours and growth was inspected. Identifications of the isolates were done by performing a series of biochemical tests and isolates of entrobacteriaceae were sub-cultured onto Hicrome ESBL agar base for phenotypic detection of ESBL producing species. Antimicrobial susceptibility tests were done for other commonly used antibiotics following disk diffusion method (29,30)

5.8.2.2. Identification

ESBL producing entrobacteriaceae was identified based on colour of colonies. That is E.coli produces pink or purple colonies. ESBL producing members of the entrobacteriaceae such as *Klebsiella, Enterobacter, Serratia, Citrobacter* species produce bluish green colonies and *Proteus, Morganella* and *Providencia* do not utilize any chromogen resulting in colourless to light brown colonies (32). Biochemical tests were performed on colonies from primary cultures for final identification of the isolates. Biochemical tests such as indole production, sugar fermentation, H_2S production, gas production, citrate utilization, motility test, urease test, oxidase were used to identify entrobacteriaceae isolates up to species (30).

Susceptibility testing was performed on isolates based on the agar disk diffusion techniques. That is from a pure culture 3-5 selected colonies of bacteria were taken and transferred to a tube containing 5ml sterile nutrient broth (Oxoid) and mixed gently until a homogenous suspension was formed and incubated at 37^{O}_{C} until the turbidity of the suspension become adjusted to a McFarland 0.5 (30).

A sterile swab was dipped into the suspension of the isolate in broth, squeezed free from excess fluid against the side of bottle. The test organism was uniformly seeded over the Mueller-Hinton agar. The antimicrobial susceptibility patterns of the test organism were evaluated against. ceftazidime(30µg), ceftriaxone(30µg), cefpodoxime(10µg), cefpirome(30µg), amoxacillin with clavulanic acid (agumentin) (30µg) ciprofloxacin (5µg), norfloxacillin (10µg), sulfamethoxazol/trimethoprin (1.25/23.75µg), Gentamicin (10µg) and chloramphenicol (30µg). The medium was then incubated at 37^{o}c for 18-24 hours. Then zone of inhibition around the discs was measured to the nearest millimeter using a metal caliper, and the isolates were classified as susceptible, intermediate and resistant (31).

5.9. Quality Control

The reliability of the study finding was guaranteed by implementing quality control measures throughout the whole processes of the laboratory works. All materials, equipments and procedures were adequately controlled. Culture Medias were tested for sterility and performance. Pre-analytical, analytical and post-analytical stages of quality assurance that are incorporated in standard operating procedures of the microbiology laboratory of University of Gondar were strictly followed. *Escherichia coli* (NCTC 13351), *Klebsiella pneumoniae* (ATCC 700603) and *Candida albicans* were used in controlling the testes carried out in this study. The inoculums density of bacterial suspension for the susceptibility test was standardizing by using 0.5McFarland standard (32).

5.10. Data Analysis

Data were entered into SPSS version 20 statistical software package for analysis. The data was analyzed using descriptive statistics. The result was presented in the form of tables, figures and texts using frequencies and summary statics such as percentage to describe the study population in relation to relevant variables.

5.11. Ethical Consideration

Ethical clearance was obtained from Research and Ethical Review Committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Official permission was obtained from University of Gondar Hospital management. The purposes and the importance of the study were explained and its confidentiality was maintained at all level of the study.

6. RESULTS

6.1. Distribution of Environmental samples

In this study, a total of 384 specimens were collected from various sources, surfaces of inanimate objects and waste water from different sewage system hospital were used as shown in table1.

Table1. Distribution of environmental samples per different sites at University of Gondar Referral Hospital, Northwest Ethiopia, January to June2014.

Type of Sample	Number (%)	Site of the hospital, n(%)				
		Wards N=20	Pharmacy N=4	Laboratory N=4	OPD N=16	Cafe N=1
Bed side table	70(18.2)	57(14.8)	-	-	13(3.4)	-
Bed frame	69(18)	56(14.5)	-	-	13(3.4)	-
Floor	69(18)	56(14.5)	-	-	13(3.4)	-
Wall	69(18)	56(14.5)	-	-	13(3.4)	-
Door Handles	34(8.9)	28(7.2)	2(0.5)	2(0.5)	2(0.5)	-
Sink	22(5.7)	18(4.6)	1(0.2)	1(0.2)	2(0.5)	-
Waste water	15(3.9)	13(3.4)	-	2(0.5)	-	-
Waiting chair	31(8.1)	26(6.8)	-	-	5(1.3)	-
Cafe chair	5(1.3)	-	-	-	-	5(1.3)

6.2 Distribution of Entrobacteriaceae in the hospital environments

A total of 163(42.45%) entrobacteriaceae were isolated from the hospital environment, bed side tables, bed frames, floors, walls, door handles, sinks, waste water, waiting chairs and cafe chairs during the study period. Of all isolates, 106(65.0%) were non-ESBL producers and 57(35.0%) were ESBL producing of entrobacteriaceae. *E.coli* 82 (50.3%) was the most common isolates followed by *K.pneumoniae* 60 (36.8%), *P.mirabilis* 5(3.1%) and *E.aerogens* 5 (3.1%). The most common species of entrobacteriaceae that produce ESBL were *K.pneumoniae* (42.1%) followed by *E.coli (35.1%) and P.mirabilis* (7.0%) (Table 2).

Table2. Isolates of entrobacteriaceae in hospital environments at University of Gondar Referral Hospital, Northwest Ethiopia, and January to June2014.

Bacterial isolates	**Number N (%)**	**Status of ESBL production**	
		NON-ESBL N (%)	**ESBL N (%)**
E.coli	82 (50.3)	62(38.0)	20 (12.3)
K.pneumoniae	60 (36.8)	36(22.1)	24 (14.7)
K.ozenae	2 (1.2)	0(0.0)	2 (1.2)
P.mirabilis	5 (3.1)	1(0.6)	4 (2.4)
E.cloceae	4 (2.5)	3(1.8)	1(0.6)
E.aerogens	5 (3.1)	3(1.8)	2 (1.2)
Citrobacter	3 (1.8)	1(0.6)	2 (1.2)
Providencia stuart	2 (1.2)	0(0.0)	2 (1.2)
Total	**163(100.0)**	**106 (27.6)**	**57(14.8)**

ESBL producing entrobacteriaceae,57(14.8%) were isolated from bed side table, bed frame, sink, wastewater, waiting chair, door handles, floor and wall except cafe chair. However, Non-ESBL producing entrobacteriaceae 106(27.6%) were isolated in all selected hospital environment sample (table 3).

Table3. The distribution of entrobacteriaceae in different hospital environmental samples at the University of Gondar Referral Hospital, Northwest Ethiopia, January to June2014.

Type of Sample	Distribution of Entrobacteriaceae		
	Non ESBL Producing N (%)	ESBL Producing N (%)	Total N (%)
Floor (n=69)	22(31.9)	3(4.3)	25 (36.2)
Bed frame n=69)	20(28.9)	9(13.0)	29 (42.0)
Door Handles (n=34)	18(52.9)	1(2.9)	19(55.9)
Bed side table (n=70)	14(20)	13(18.6)	27(38.6)
Sink (n=22)	9(40.9)	13(59)	22(100.0)
Wall (n=69)	9(13)	2(2.9)	11(15.9)
Waiting chair (n=31)	4(12.9)	2(6.4)	6(19.3)
Waste water (n=15)	6(40)	9(60)	15(100)
Cafe chair (n=5)	2(40)	0(0.0)	2(1.2)
Total (n=384)	106(65.0)	57(35.0)	163(100.0)

6.2. Distribution of ESBL producing entrobacteriaceae in the hospital environment

ESBL producing entrobactewriaceae namely *E.coli, K.pneumoniae, K.ozane, P.mirabilis, E.cloceae, E.aerogens, Citrobacter* and *providencia stuarti* were isolated from the samples.Of these the predominant ESBL producing Entrobacteriaceae was *K.pneumoniae*, 24(4.7%) followed by *E.coli*, 20(12.3%) and *P.mirabilis* 4(2.4%). *E.coli* 20(12.3%) and *K.pneumoniae* 24(14.7%) were distributed in all selected type of samples. However, *K.ozenae* 2(1.2%) in sink, *P.mirabilis* 4(2.4%) in bed frame, floor & sink, *E.cloace*, 1(0.6%) in bed frame, *E. aerogens*, 2(1.2%) in bed table & bed frame, *Citrobacter*, 2(1.2%) in floor & sink and *P.stuarti*, 2(1.2%) in bed frame & sink were found (Table3).

Of the 57 ESBL producing entrobacteriaceae, the highest number of isolates were recovered from waste water 9 (60%), sink 13(59%), and bed table 13(18.6%). ESBL producing organism found in bed frame, floor, wall, cafe chair, door handles waiting chair are less than 5% each. However, ESBL producing entrobacteriaceae were not isolated from hospital cafe chairs (table3).

Table4. Profile of ESBL producing entrobacteriaceae in different hospital environmental samples at University of Gondar Referral Hospital, January to June 2014.

ESBL producing entrobacteriaceae	**Type of sample**								
	Bed side table N=70	Bed frame N=69	Floor N=69	Wall N=69	Door handler N=34	Waiting Chair N=31	Sink N=22	Waste water N=15	Cafe Chair N=5
E.coli	3 (4.3)	3 (4.3)	1 (14)	1 (1.4)	1 (2.9)	1 (3.2)	3 (13.6)	7 (46.7)	0 (0.0)
K.pneumoniae	8 (11.4)	2 (2.9)	1 (1.4)	1 (1.4)	0 (0.0)	1 (3.2)	4 (18.1)	7 (46.7)	0 (0.0)
K.ozenae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.5)	0 (0.0)	0 (0.0)
P.mirabilis	1 (1.4)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (9.0)	0 (0.0)	0 (0.0)
E.cloace	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
E.aerogens	1 (1.4)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Citrobacter	0 (0.0)	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)
p.stuart	0 (0.0)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
Sum=57	13 (22.8)	9 (13.0)	3 (4.3)	2 (2.9)	1 (2.9)	2 (3.5)	13 (59.0)	14 (93.3	0 (0.0)

The highest proportion of ESBL producing entrobacteriaceae were isolated from sewage 14(93.3%) followed by medical ward 30 (15.9%) and surgical ward 6 (11.5%) (Table5).

Table5. Distribution of ESBL producing Entrobacteriaceae in different sites of hospital environment at University of Gondar Referral Hospital, Northwest Ethiopia, January to June2014.

Bacterial isolates	Site of environmental sample						
	Medical Ward N=188	**Surgical ward N=70**	**Gyn-obs ward N=52**	**Fistula clinic N=29**	**Eye Clinic N=25**	**Sewage N=15**	**Cafe N=5**
E.coli	12 (6.3)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	7 (46.7)	0 (0.0)
K.pneumoniae	14 (7.4)	1 (1.4)	1 (1.9)	0 (0.0)	1 (4.0)	7 (46.7)	0 (0.0)
K.ozenae	1 (0.5)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
P.mirabilis	2 (1.0)	1 (1.4)	0 (0.0)	1 (3.4)	1 (4.0)	0 (0.0)	0 (0.0)
E.colace	0 (0.0)	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
E.aerogens	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)	0 (0.0)	0 (0.0)
Citrobacter spp	1 (0.5)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
P.stuart	0 (0.0)	1 (1.4)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	30 (15.9)	6 (8.6)	3 (5..8)	1 (3.4)	3 (12.0)	14 (93.3)	0 (0.0)

6.3. Antimicrobial susceptibility patterns of ESBL producing entrobacteriaceae isolates from the hospital's environment

The results of antimicrobial susceptibility pattern of isolates were summarized in table 6. All ESBL producing Entrobacteriaceae were 100% resistant to cefpirome, cefpodoxime, ceftazidime, ceftriaxone & amoxacilin with clavulanic acid. Moreover 40(70.2 %), 37(64.9 %), 24(42.1%), 25 (43.9%), 11(19.1%) were found to be resistant to chloramphenicol, cotrimoxazole, norfloxacillin, ciprofloxacillin and gentamicin respectively. Gentamicin was relatively effective against ESBL producing entrobacteriaceae among non betalactam antibiotics (Table 6).

All isolated *K.pneumoniae* and *E.coli* were resistant to cefpirome, cefpodoxime, ceftazidime, ceftriaxone and amoxacilin with clavulanic acid. Moreover, majority of K.pneumoniae was resistant to cotrimoxazole 22 (91.7%), chloramphenicol 16 (66.7%), norfloxacilin 11 (45.8%), ciprofloxacilin 11(45.8%) and gentamicin 6(25.0%). E.coli was also resistant to chloramphenicol 11(25.0%).cotrimoxazole 7(35.0%), norfloxacilin 5(25.0%), ciprofloxacilin 6(30.0%), and gentamicin 4(20.0%).

All isolates of *P. mirabilis* were resistant to cefpirome, cefpodoxime, ceftazidime; ceftriaxone, augmentin and chloramphenicol. Of the total of 4 ESBL producing *P.mirabilis*, only one strain was resistant to norfloxacilin , ciprofloxacilin and cotrimoxazole . However, all isolates of *P.mirabilis* were susceptible to gentamicin .

All isolates of *K. ozenae* were resistance cefpirome, cefpodoxime, ceftazidime; ceftriaxone, amoxacillin with clavulanic acid, chloramphenicol and cotrimoxazole. However, all isolates of *K.ozenae* were sensitive to norfloxacilin, ciprofloxacilin and gentamicin.

Entrobacter cloceae and *Entrobacter aerogens* showed resistance 1(100%) and 2(100%) to cefpirome, cefpodoxime, ceftazidime; ceftriaxone, amoxacilin with clavulanic acid, ciprofloxacilin, norfloxacilin, chloramphenicol and cotrimoxazole, whereas 1(100%) and 2(100%) of the isolates of *E.cloceae* were sensitive to gentamicin.

All isolates of *citrobacter* were resistant to cefpirome, cefpodoxime, ceftazidime ceftriaxone, augmentin, chloramphenicol, cotrimoxazole, norfloxacilin, ciprofloxacilin and all isolates of *citrobacter* were sensitive to gentamicin.

Providencia stuart showed high level of resistance to 2(100%) cefpirome, cefpodoxime, ceftazidime, ceftriaxone, amoxacilin with clavulanic acid, cotrimoxazole, norfloxacilin, ciprofloxacilin and chloramphenicol and 1(50.0%) isolates of *p.stuart* were sensitive to gentamicin.

Table6: Antimicrobial susceptibility pattern of ESBL producing entrobactereceae isolated from hospital environment at University of Gondar Referral Hospital, Northwest Ethiopia, January to June2014.

Organism	Pattern	Antimicrobial agent's n (%)									
		CFP	CPD	CAZ	CTR	CIP	NX	GEN	C	AMC	SXT
E.coli	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (70.0)	15 (75.0)	16 (80.0)	9 (45.0)	0 (0.0)	13 (65.0)
	R	20 (100)	20 (100)	20 (100)	20 (100)	6 (30.0)	5 (25.0)	4 (20.0)	11 (55.0)	20 (100)	7 (35.0)
K.pneumonea	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	13 (54.2)	13 (54.2)	18 (75.0)	8 (33.3)	0 (0.0)	2 (8.3)
	R	24 (100)	24 (100)	24 (100)	24 (100)	11 (45.8)	11 (45.8)	6 (25.0)	16 (66.7)	24 (100)	22 (91.7)
K.ozenae	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	2 (100)	2 (100)	2 (100)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	2 (100)	2 (100)
P.mirabilis	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (75.0)	3 (75.0)	4 (100)	0 (0.0)	0 (0.0)	2 (50.0)
	R	4 (100)	4 (100)	4 (100)	4 (100)	1 (25.0)	1 (25.0)	0 (0.0)	4 (100)	4 (100)	2 (50.0)
E.cloace	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)
	R	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.0)	1 (100)	1 (100)	1 (100)
E.aerogens	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)
	R	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0 (0.0)	2 (100)	2 (100)	2 (100)
Citrobacter	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)
	R	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0 (0.0)	2 (100)	2 (100)	2 (100)
P.stuart	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50.0)	2 (100)	2 (100)	2 (100)
Total(57)	S	0 (0%)	0 (0%)	0 (0%)	0 (0%)	32 (56.1%)	33 (57.9%)	46 (80.7%)	18 (31.6%)	0 (0.0%)	17 (29.8%)
	R	57 (100%)	57 (100%)	57 (100%)	57 (100%)	25 (43.9%)	24 (42.1%)	11 (19.3%)	39 (68.4%)	57 (100%)	40 (70.2%)

CFP- cefpirome, CPD-cefpodoxime, CAZ- ceftazidime, CTR- ceftriaxone, CIP-ciprofloxacilin, NX- norfloxacilin, GEN- gentamicine, C- chloramphenicol, AMC-augmentin, SXT- Sulfamethoxazol/Trimethoprime, S-Sensitive and R- Resistance.

6.4. Multiple antibiotics resistant

More than fifty six percent of ESBL producing entrobacteriaceae isolates were demonstrated multiple non beta lactam antibiotics resistance against ciprofloxacilin, norfloxacilin, gentamicin, chloramphenicol, amoxacillin with clavulanic acid and cotrimoxazole.The most com,mon multiple drug resistance isolates were *K .pneumonia* 14(58%) and *E.coli* 8(40%). Despite the number of isolates are small isolates, *E.cloceae*, *E.aerogens,Citrobacter* and *P.stuarti* were multidrug resistance (Table 7).

Table7. Multiple non-beta-lactam antibiotic resistance pattern of ESBL-producing entrobacteriaceae isolated from hospital environment at Gondar University Referral hospital, Northwest Ethiopia, January to June2014.

ESBL producing entrobacteriaceae	Total	Antibiogram pattern						
		R0	**R1**	**R2**	**R3**	**R4**	**R5**	**MDR**
E. coli	20	8(40)	4(20)	3(15)	2(10)	0(0)	3(15)	8(40)
K. pneumonia	24	2(8.3)	8(33.3)	3(12.5)	6(25)	2(8.3)	3(12.5)	14(58)
K. ozenae	2	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)	0(0.0)	2(100)
P.mirabilis	4	0(0.0)	3(75)	0(0.0)	0(0.0)	1(25)	0(0.0%)	1(25)
E. clocea	1	0(0.0)	0(0.0)	00.0)	0(0.0)	1(100)	0(0.0%)	1(100)
E.aerogens	2	0(0.0)	0(0.0)	0(0.0)	1(50)	1(50)	0(0.0)	2(100)
Citrobacter	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)	2(100)
P. stuart	2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)	0(0.0)	2(100)
Total	57(14.84)	10(1.8)	15(26.3)	8(14.0)	9(15.8)	9(15.8)	6(10.5)	32(56.1)

R0 = All susceptible, R1-R5 = Resistance to 1-5 antibiotics respectively

MDR= Multiple Drug Resistant

7. DISCUSSION

This study demonstrates the presence of ESBL producing entrobacteriaceae at the University of Gondar Referral Hospital environment. In many developing countries, including Ethiopia, routine ESBL detection is not commonly carried out. Thus, assessment of the distribution of the ESBL producing bacteria is very important to develop guideline in the management of infections associated with such organisms.

According to the present study, the total entrobacteriaceae were 42.5%. The predominant isolate was *E.coli* (50.3%) and *Klebsiella pneumonia* (36.8%), this is higher than the study done in Nigeria *E.coli* (7.15%), *Klebsiella pneumonia* (5.3%). This may be due to difference in type of health care activities and infection control practice. However, the prevalence of *Proteus mirabilis* (3.1%) and *Entrobacter aerogens* (3.1%) are in line with the study done in Nigeria (3.5%) and (3.5%), respectively (16). Moreover, the result of the present study is higher than studies from Ethiopia, Gondar Hospital, *Klebsiella pneumonia* (26.8%), *Escherichia coli* (24.3%), but lower in *Entrobacter* s*pp.* (9.8%) and *Proteus spp.* (12.2%) (17). Although, the study area is the same, there is a discrepancy in the result this may be due to difference in sample size and type of sample, season of collection and type of patients who attend to the hospital.

The total isolates of Enterobacteriaceae from waste water were 24.6%, being the only isolates were *K.pneumoniae* (12.3%) and *E.coli (12.3%).* The same area, which was done in sewage system of the University of Gondar hospital showed that high prevalence of *K. pneumoniae* (22.9%) and *E.coli* (10.4%) (24). This result variation may be due to improvement of sanitation system in the hospital.

Regarding the ESBL producing entrobacteriaceae, the predominant ESBL producing species was K. *Pneumoniae* (14.7%). This is in line from studies in Alexanderia, Egypt (14.9%) (28) but, lower than the study done in France (37%) and Algeria (44.5%) (18, 21) and Upper Egypt (56.25%) (22,). This result variation may be due to the way of infection prevention activities and the overall sanitation activities of the sites.

The second predominant ESBL producing organism was *E. coli* (12.3%) which was higher than the study done in France (5%), Algeria (4%) (18, 21). However, this finding is lower as compared to the reports from Alexanderia, Egypt (85%) and Upper Egypt (43.75%) (28, 22).These result differences may be due to difference in type of health care activities and infection control practice in the hospital. *E. cloaca* (1.2%) is lower than Algeria's finding in intensive care unit of the hospital (11%) (21)

Another study which was done in Egypt, Zagazig University Hospital, showed the distribution of ESBL producing entrobacteriacae, specifically *K.pneumoniae* 2.3%. if the type of sample is different to the current study, the prevalence is lower than the current finding (14.7%) (23).

According to the study, the inanimate objects in hospital OPD (Out Patient Department), wards, surgery room, Delivery room, diagnosis section, waiting area and waste water in different sewage system of the hospital were variously contaminated by multiple drug resistance ESBL producing bacteria. Moreover sewage 14(93.3%) had high rate of ESBL producing entrobacteriaceae followed by medical department 30(15.9%), Eye clinic 3 (12.0%), Surgery 6 (8.6%), Gynacology & Obstetrics 3 (5.8%), and Fistula clinic 1 (3.4%). The overall distribution of ESBL producing Enterobacteriaceae in different section was higher than the report in France (18). This may be due to the amount of patient attends in each section. The value of ESBL producing organism in different sites is directly related to the type of sample.

When observing the current study and other studies, hospital environment contamination is caused mostly by ESBL producing *K.pneumoniae* than ESBL producing *E.coli* in entrobacteriaceae family (19). Different studies show that the proportion of organism which causes environmental contamination is directly associated to the patient cases and other studies said that the transmission rate of ESBL producing *K.pneumoniae* is higher than the ESBL producing *E.coli*. So in the current study most patient cases can be related to *K.pneumoniae* because it is the highest ESBL organism (18.19, 20).

The antimicrobial susceptibility test showed different percentage of resistance among ESBL producing entrobacteriaceae. All isolates of ESBL producing entrobacteriaceae were 100% resistant to cefpirome, cefpodoxime, ceftazidime, ceftriaxone & amoxicillin with clavulanic acid. Moreover. the present study was much higher than reports from Poland cefpodoxime (73.5%), and ceftazidime (81.6%) (26) and Upper Egypt, *K.pneumoniae* (95.5%) for ceftazidime and *E.coli* (91.4%) for ceftazidime (20).

Even though the rate of resistance was low for non beta lactam antibiotics compared to beta lactam antibiotics, ESBL producing entrobacteriaceae demonstrated alarming rate of resistance, which can serve as an alternative choices for resistant strains. More than half of *K.pneumoniae* and *E.coli* were resistant to chloramphenicol ,cotrimoxazole, norfloxacilin, gentamicin and ciprofloxacilin whish were commonly used antibiotics. *K.pneumoniae* developed resistant rates of 25.0% and 45.8% for gentamicin and ciprofloxacilin, respectively, which was lower than studies in Upper Egypt (84.4%, gentamicin and 77.7% ciprofloxacilin) (20). *E.coli* isolates also showed almost the same pattern of resistance 20% for gentamicin and 30% for ciprofloxacilin. Low isolation rate compared to similar study in Upper Egypt (42.8% gentamicin and 68.5% ciprofloxacilin) (20).

The most challenging things in management of infectious disease associated with ESBL producing entrobacteriaceae are development of multiple drug resistant (Resistant to two or more drugs). There is different report of co-resistance in case of ESBL producing entrobacteriaceae, but it is not more than three antibiotics (22). However, this study shows high frequency of multiple antibiotics resistance to the commonly used antibiotics. This might be a reflection of in appropriate use of antimicrobials, lack of laboratory diagnostic tests, unavailability of guideline for the selection of antibiotics, that is, some antibiotics are commonly used.

8. LIMITATION OF THE STUDY

The limitation of this study was that ESBL producers were isolated only by phenotypic method. It would have been good if the genetic type of specific resistance was identified using molecular technique but due to shortage of material and resource it was not done in this study.

9. CONCLUSION

The result showed that 14.8% of entrobacteriaceae found in the hospital environment were ESBL producing which pose a risk of its spread to the environment and subsequent spread to human and animal exposure. The predominant ESBL producing entrobacteriacae were found to be *K.pneumonae, E.coli, P.mirabilis*. In waste water, sink, and bed tables higher number of ESBL producing entrobacteriaceae were isolated. Higher proportion of ESBL producing entrobacteriaceae isolates were also found from sewage. All ESBL producing entrobacteriaceae were resistant to ceftraxone, ceftazidime, cefpirome, cefpodoxime and amoxacillin with clavulanic acid. Those ESBL producing isolates were also showed resistance to non beta lactam antibiotics like 40(70.2%) chloroamphenicol, 37(64.9%) cotrimozazole, 24(42.1%) norfloxacilin, 25(43.9%) ciprofloxacilin and 11(19.1%) gentamicin.

10. RECOMENDATION

- Proper way of infection prevention and control practices should be exercised in the hospital environment and good hygiene activities should continuously monitored in hospital.
- Sewage system of the hospital should be properly practice to prevent environment contamination.
- Rational use of antibiotics should carefully practice
- Future studies are needed to identify ESBL producing entrobacteriaceae at molecular label.

-

11. REFERENCES

1. Prescott LM, Harley JP, Klein DA. Microbiology. McGraw-Hill. NewYork.6th ed. 2005: 833-842.
2. Hawkey PM, Jones AM. The changing epidemiology of resistance. *J. Antimicrob Chemother* 2009;64(1):3-10
3. Pitout JD, Laupland KB, Extended-spectrum beta lactamase-producting Entrobacteriaceae: an emerging public-health concern.. *Lancet Infect Dis* 2008:8:159-66.
4. Naas T, Poires L, Nordmann P. 2008. Minor extended-spectrum β-lactamase. *Clin Microbiol Infect.* 2008; 14(1):42-52.
5. Canton R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM. Prevalence and spread of extended-spectrum β-lactamase-producing entrobacteriaceae in Europe. *Clin Microbiol Infect*. 2008; 14(1):144-153
6. Paterson DL, Bonomo RA. Extended spectrum β-lactamases: a clinical update, *Clin Microbiol Rev*. 2005; 18(4):657-86.
7. Pitout JD, Nordmann P, Kevin B, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *J Antimicrob Chemother*. 2005;56:52–9.
8. Rafay AM, Al-Muharrmi Z, Toki R. Prevalence of extended spectrum β-Lactamases producing isolates over a 1 year period at a University Hospital in Oman. *Saud Med* J. 2007; 28(1): 22–27.
9. Ben-Ami R, Schwaber MJ, Navon-Venezia S, et al. Influx of extended-spectrum β-lactamase-producing Enterobacteriaceae into the hospital. *Clin Infect Dis* 2006; 42(1):925-934.
10. Livermore DM, Canton R, Gniadkwski M, Nordmann P, Rossolini GM, et al. CTX-M. changing the face of ESBLs in Europe. *J Antimicro Chemother*. 2007; 59(1):165-
11. Carattoli A. Animal reservoirs for extended-spectrum β-lactamase producers. *Clin Microbiol Infect.* 2008; 14: 117-123.
12. Sturenburg E, Mack D. Extended-spectrum β-lactamases implications for the clinical microbiology laboratory, therapy and infection control. *J Infect* 2003; 47(4):273-95.

13. Al-Jasser AM. Extended spectrum beta lactamases (ESBLs): A global problem. *Kuwait Med J.* 2006; 38(3):171-85.
14. Livermore DM, Woodford N. The β-lactamase threat in Entrobacteriaceae, Pseudomonas and Acinetobacter. *Tren in Microbiol*. 2006; 14(1):413-414.
15. Bonnet R, Growing group of extended-spectrum beta-lactamases. The CTX-M enzymes. *Antimicro Agents Chemothe*. 2004; 48(1):1-14.
16. Chikere C, Omoni V, Chikere B. Distribution of potential nosocomial in a Hospital environment. *African J of Biotec.* 2008; 7(20):3535-3559
17. Gelaw A, Gereselassie S, Tiruneh M, Matiwos E, Yifru S. Isolation of bacterial pathogens from patients with postoperative surgical site infection and sources of infections at the University of Gondar Hospital Northwest Ethiopia. *J Enviro Occcup* Sci.2014; 3(2):103-108.
18. Guet-Revillet H, Le Monnier A, Breton N, et al. Environmental contamination with extended-spectrum beta-lactamses: is there any difference between Escherichia coli and Klebsiella spp? *Am. J Infect Control* 2012; 40(9): 845-848.
19. Freeman JT, Nimmo J, Gregory E,Tiong A, Almeida MD, Mcauliffe GN, Roberts SA.Predictors of hospital surface contamination with Extended-spectrum beta lactamase producing *Eschericha coli* and *Klebsiella pneumonia*:patient and organism factors.*Antmicro Resis and Infct cont*.2014;3(5).
20. Magdy M. Affi. Detection of Extended Spectrum Beta-lactamase producing Klebsiella pneumonia and Escherichia coli of environmental surfaces at Upper Egypt. *Int. J. Biol. Chem.* 2013; 16 (2): 212-231
21. Baba Ahmed Z, Ayad A, Mesli E, Messai Y, Bakour R and Drissi M. CTX-M-15 extended-spectrum β-lactamases in Entrobacteriaceae in the intensive care unit of Tlemcen Hospital, Algeria. *Est. Medit. Health J.* 2002; 18(4):382-386.
22. Gbaguidi-Haore H, Talon D, Hocquet D and Bertrand X. Hospital environmental contamination with Enterobacteriaceae producing extended-spectrum β-lactamse. *Am.J Infect Control* 2013; 12(4):56-16.
23. Abdalla A. El-Bialy, Ali Abu-Zeid. Prevalence and risk factors of Extended-spectrum B-lactamase producing Kelbesiella pneumonia in Neonatal intensive care

unit. Egypt.J. of med. Microbiol. 2009: 18(1).110-115.

24. Moges F, Endris M, Belyhun Y, Worku W. Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environments, Northwest Ethiopia. *Bio Med Central.*2014
25. Monaghan C and Colleran E. "Antibiotic resistance of faecal coli forms in hospital and city sewage in Galway. *Ir J Med. Sci*. 1981; 150(10). 304-309.
26. Esiobu N, Armenta , Lke J. "Antibiotic resistance in soil and water environments''. *Int J Environ Health Res.2002.* 12 (2). 133-144.
27. Korzeniewska E, Harnisz M. Beta-lactamse-producing Entrobacteriaceae in hospital effluents. *J Envir. Manage*. 2013; 128: 904-11.
28. Amira E,K.Amine. Extended spectrum beta lactamase producing Bacteria in waste water Alexandria, Egypt. *Int.J. Biosci. Biochem and Bioinform*. 2013; 3(6). 285-290.
29. Glupczynski Y, Berhin C, Bauraing C, & Bogaert P. valuation of a new selective chromogenic agar medium for detection of extended spectrum beta lactamase producing entrobacteriaceae. *J Clin Microbiol*. 2007; 45 (2):501-505.
30. Cheesbrough M. Mannual of medical microbiology. 2nd ed, Britain, oxford press; 2006; 63-143**.**
31. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by standard single disc method. *Ameri J. Clin Patho*.1996; 45(45):493-6.
32. Arora D. Quality assurance in microbiology. *Indian J Med Microbiol.* 2013.17 (22): 81-6.
33. Clinical and Laboratory standards Institute (CLSI) performance standards for antibiotic susceptibility testing twenty third informational supplement CLSI document M100-S23. Wayne, penssylavia USA: Clinical and Laboratory standaeds Institute 2013.

12. ANNEXES

12.1. Media and biochemical test preparation

A. Hicrome Agar base

HiCrome ESBL Agar Base is chromogenic screening medium for the selective isolation of ESBL producing organisms. It contains peptone mix and yeast extract, which serves as the carbon and nitrogen sources. Chromogenic mixture is used to differentiate the ESBL producing organisms on the basis of colour. HiCrome ESBL Selective Supplement (FD278) helps in inhibition of other contaminating organisms. ESBL producing *E.coli* grows as either pink or purple colonies. ESBL producing members of the KESC group produce bluish green colonies; *Proteus, Morganella* and *Providencia* do not utilize any chromogen resulting in colourless to light brown colonies. The media prepared by suspend 40 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely, sterilize by autoclaving at 15lbs pressure (121^{o}_{c}) for 15 minutes, Cool to 50^{O}_{C} and add rehydrated contents of two vials of Hicrome ESBL selective supplement (FD278). Mix well and pour into sterile plates (28).

B. Mueller Hinton Agar preparation

Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to cefpirome(30µg), ceftazidime(30µg),cefpodoxime (10µg) and ceftriaxone (30µg), And also ciprofloxacin (5µg), sulfamethoxazol/trimethoprim (1.25/23.75µg), norfloxacin (5µg) and gentamicin(10 µg), chloramphenicol (30µg). Muller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method and the zone of inhibition is considered as the minimum concentration that inhibits the target organism.

C. Preparation of biochemical tests

Biochemical tests are used to differentiate different organisms based on their genus and species characteristics. Biochemical tests are performed on pure culture. At time of prepared the sterility should be checked. Biochemical tests are TSI, MIU, Simmon Citrate and Urea.

12.2. Data collection form

A. Sample is inanimate sample

1. Source (object) where sample taken ----------

   Specific ward.....

2. Code number----------

3. Media used ----------

4. Organism isolated ----------

5. Drug susceptibility pattern

   5.1. Sensitive to ----------

   5.2. Resistance to----------

   5.3. Intermediate to----------

6. Other remarks ----------

B. Waste water from hospital sewage

1. Source where sample taken.....

2. Code number.....

3. Media used.....

4. Organism isolated.....

5. Drug susceptibility.....

5.1. Sensitive to.....

5.2. Resistance to.....

5.3. Intermediate to.....

6. Other Remarks.....

12.3. Collection and processing of environmental samples

A. Collection of Sample in inanimate objects

1. Environmental samples was taken from inanimate objects in Intensive Care unit, medical, orthopedic, gynecologic, obstetric, ophthalmologic, pediatric, emergency room, reception area, Laboratory, Pharmacy and café room.

2. Using sterile cotton tipped applicator sticks moistened with normal saline collect sample from the surface of the object.

3. Role the swab over the surface of object on 10 by 10cm square was sampled with five longitudinal and five latitudinal (19).

4. Label the sample as soon as possible with the name of the site and type of inanimate object.

5. Inoculate the specimen in to prepared MacConkey media aseptically.

6. Inoculate the growth colonies in to biochemical test to differentiate the species

7. Then inoculate the growth colonies in to prepared Hicrome agar base aseptically to know whether the organism is ESBL or not.

8. Incubate the plate aerobically at 35-37 $^{o}_{C}$ for 24-48 hours.

9. Examine and report the culture; look for colony characteristics (color of colonies) and perform biochemical test (MIU, Simmon citrate, SIM and TSI).

10. Determine drug susceptibility pattern of the isolated organism.

B. Collection of waste water from sewage of the hospital

1. Waste water was collected from hospital associated sewage.

2. The waste water was collected using sterile container and label the site of collected

3. Inoculate loop full waste water in to prepared MacConkey media aseptically.

4. Inoculate the growth colonies in to biochemical test to differentiate the species

5. Then inoculate the growth colonies in to prepared Hicrome agar base aseptically to know whether the organism is ESBL or not

6. Inoculate the plate aerobically at 35-37oc for 24-48 hr.

7. Examine and report the culture; look for colony characteristics (color of colonies) and perform biochemical test (MIU, Simmon citrate, SIM and TSI).

8. Determine drug susceptibility pattern of the isolated organism.

C. Biochemical testing procedure

Extended spectrum beta lactamase producing entrobacteriaceae species is identified based on glucose and lactose fermentation, H2S production, indole formation from tryptophan, production of acetylmethyl carbinol from glucose, liquification of gelatin, use of citrate as a carbon source, Hydrolysis of urea and decarboxylation of amino acids. MIU, Simmon citrate, TSI and biochemical test was used.

1. Motility Indole Urea (MIU)

Motility indole urea is a composite medium containing tryptone, phenol red, urea and a paper strip moistened in kovac's reagent. It used to differentiate entrobacteriaceae by urea, motility and indole characteristics**.**

Procedure

1. Prepared MIU and dispense in sterile test tube and incubate at 37oc over night.

2. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth.

3. Take sample from the suspension by straight wire.

4. Inoculate by straight wire through the centre of the medium.

5. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours.

6. Interpretation:

- Non-motile organism grow only in the line of the inoculums,
- Motile organism grow throughout the medium which become turbid
- Urease positive organism turns the medium red
- Indole positive organisms turn the Kovac's strips red

1. Simmon Citrate

This test is used to differentiate species of the family enterobacteriaceae. It is selective for bacteria that have the ability to consume citrate as its sole source of carbon and ammonium as sole nitrogen source.

Procedure

1. Prepare slopes of the medium in the sterile test tube

2. Using a sterile straight wire, first streak the slope with suspension of the test organism and then stab the butt.

3. Incubate at 35-37oc for 48 hours

4. Interpretation

Bright blue----------positive citrate test (*klebsiella,protus,salmonella,citrobacter species)*

No change in color of medium-------Negative citrate.

2. Triple Sugar Iron (TSI)

Used for differentiation of entrobacteriaceae by observing fermentation of glucose, lactose and sucrose, H2S and gas production.

Procedure

1. Prepared TSI, dispense in sterile test tube and make slant then incubate at 37oc over night.
2. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth.
3. Take sample from the suspension by wire loop.
4. Inoculate by wire loop, first streak the slope with suspension of the test organism and then stab the butt.
5. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours.
6. Interpretations:

- Slant color red …… does not ferment either lactose or sucrose
- Slant color yellow…….Ferments lactose and/or sucrose
- Butt color red……no fermentation of glucose
- Butt color yellow……. fermentation of glucose and acid produced
- Cracks in media ……Gas formed bubbles or entire slant Pushed out of the tube.
- Blackening …. ……H_2S has been produced

D. Antimicrobial sensitivity testing

A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of sensitivity testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from a disc that

is related to the sensitivity of the organism. Strains sensitive to the antimicrobial agent are inhibited at a distance from the disk where as resistant strains have smaller zones of inhibition or grow up to edge of the disc. The zone of inhibition is measured by clippers or ruler and values are matched (compared) with the predetermined standard values and reported as susceptible, Intermediate and resistant (32).

Procedure

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of nutrient broth.
2. Much the turbidity of suspension with turbidity standard
3. With a sterile swab take sample from the suspension.
4. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab
5. Using a sterile forceps or needle ,place the antimicrobial disc on the inoculated plate
6. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
7. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
8. Interpret the reaction:

   Sensitive ..... zone of radius is wider or equal to the control

   Intermediate ..... zone of radius is more than three mm smaller than the control

   Resistance ..... no zone of inhibition.